SANITIZED

CBIC Control Number
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TSCA Confidential Business Information Center (7407M)
WJC East; Room 6428; Attn: Section 8(e)
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

February 19, 2020

TSCA 8(e) Substantial Risk Notice for Urethane Resin

Dear Sir or Madam,

{ } previously submitted preliminary results from a mouse Local Lymph Node Assay (LLNA, OECD Guideline 429) a complex urethan resin reaction product. Please refer to docket # 8EHQ-19-21861.

The final report for that study is now available and attached.

The specific chemical identity of the test material and the identity of the submitter are considered to be confidential business information (CBI). Please see the attached CBI substantiation and certification.

Sincerely,

{

}

Enclosure (2)

Sanitized

charles river

FINAL REPORT

Test Facility Study No. [redacted]

Sponsor Reference No. [redacted]

Assessment of Skin Sensitization to [redacted] in the Mouse (Local Lymph Node Assay)

SPONSOR:

[redacted]

TEST FACILITY:
Charles River Laboratories Den Bosch BV
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

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QUALITY ASSURANCE STATEMENT

Study title: Assessment of Skin Sensitization to [redacted] in the Mouse (Local Lymph Node Assay).

This report was inspected by the Test Facility Quality Assurance Unit (QAU) according to the Standard Operating Procedure(s). The reported method and procedures were found to describe those used and the report reflects the raw data. The Test Facility inspection program was conducted in accordance with Standard Operating Procedure. During the on-site process inspections, procedures applicable to this type of study were inspected.

The dates of Quality Assurance inspections are given below.

Test Facility Study No. [redacted]

Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date to TFM and SD*
Study	Final Study Plan	24-Jul-2019	24-Jul-2019	24-Jul-2019
	Report	14-Oct-2019	15-Oct-2019	15-Oct-2019
	Final Report	23-Oct-2019	23-Oct-2019	23-Oct-2019
Process	**Animal Facilities**	10-Jul-2019	24-Jul-2019	01-Aug-2019
	Test Item Handling			
	Exposure			
	Observations/Measurements			
	Specimen Handling			
	Test Item Receipt	12-Aug-2019	22-Aug-2019	22-Aug-2019
	Test Item Handling			
	Analytical and physical chemistry	12-Aug-2019	27-Aug-2019	29-Aug-2019
	Test Item Handling			
	Exposure			
	Observations/Measurements			
	Specimen Handling			
	Test Item Formulation	15-Aug-2019	23-Aug-2019	30-Aug-2019
	Test Item Handling			

*TFM=Test Facility Management SD = Study Director

Date: 06 February 2020

Bart Kluskens, BSc
Quality Assurance Auditor

COMPLIANCE STATEMENT AND REPORT APPROVAL

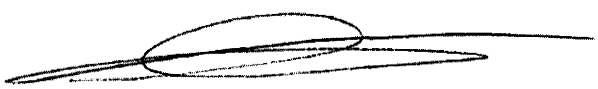
The study was performed in accordance with the OECD Principles of Good Laboratory Practice as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA and EPA), Japan (MHLW, MAFF and METI) and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions from the above regulations are listed below.

- Concentration, stability, and homogeneity of test item formulations were not determined in this study. However, to limit the impact, the test item preparation was performed with approved procedures and documented in detail. Preparations were visually inspected for homogeneity prior to use and all preparations were used within 4 hours after preparation of the formulation.

This study was conducted in accordance with the procedures described herein. All deviations authorized/acknowledged by the Study Director are documented in the Study Records. The report represents an accurate and complete record of the results obtained.

There were no deviations from the above regulations that affected the overall integrity of the study or the interpretation of the study results and conclusions.



Date: 06 feb 2020

A.H.B.M. van Huygevoort, MSc

Study Director

1. RESPONSIBLE PERSONNEL

1.1. Test Facility

Study Director	A.H.B.M. van Huygevoort, MSc
Test Facility Management	H.H. Emmen, MSc

1.2. Sponsor

Sponsor Representative / Study Monitor	[redacted]

2. SUMMARY

The objective of this study was to evaluate whether [redacted] induces skin sensitization in mice after three epidermal exposures under the conditions described in this report.

The study was carried out based on the guidelines described in:

- OECD, Section 4, Health Effects, No.429 (2010).
- EC No 640/2012, Part B: "Skin Sensitization: Local Lymph Node Assay".
- EPA, OPPTS 870.2600 (2003) "Skin Sensitization".

Test item concentrations selected for the main study were based on the results of a pre-screen test.

In the main study, three experimental groups of five female CBA/J mice were treated with test item concentrations of 10, 25 or 40% w/w on three consecutive days, by open application on the ears. Five vehicle control animals were similarly treated, but with the vehicle alone (N,N-dimethylformamide (DMF)). Three days after the last exposure, all animals were injected with ^{3}H-methyl thymidine and after five hours the draining (auricular) lymph nodes were excised and pooled for each animal. After precipitating the DNA of the lymph node cells, radioactivity measurements were performed. The activity was expressed as the number of disintegrations per minute (DPM) and a stimulation index (SI) was subsequently calculated for each group.

All auricular lymph nodes of the test item treated animals were enlarged, compared to the controls. The largest auricular lymph nodes were found in the higher dose groups. No macroscopic abnormalities of the surrounding area were noted for any of the animals.

Mean DPM/animal values for the experimental groups treated with test item concentrations 10, 25 and 40% were 4838, 8691 and 18271 DPM, respectively. The mean DPM/animal value for the vehicle control group was 602 DPM. The SI values calculated for the test item concentrations 10, 25 and 40% were 8.0, 14.4 and 30.4, respectively.

These results show that the test item elicits a SI ≥ 3. The EC3 value (the estimated test item concentration that will give a SI =3) was established to be between >0 and 10%. No reliable EC3 value could be calculated by the method of Ryan et al. (2007) because the lowest SI value (SI of 8.0) does not approach the SI=3 value.

The six-month reliability check with Alpha-hexylcinnamaldehyde indicates that the Local Lymph Node Assay as performed at Charles River Den Bosch is an appropriate model for testing for contact hypersensitivity (see Appendix 3).

Based on these results:

- According to the recommendations made in the test guidelines (including all amendments), [redacted] would be regarded as skin sensitizer.
- According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2017) (including all amendments), [redacted] should be classified as skin sensitizer (Category 1).
- According to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of items and mixtures (including all amendments), [redacted] should be classified as skin sensitizer (Category 1) and labeled as H317: May cause an allergic skin reaction.

3. INTRODUCTION

The objective of this study was to evaluate whether [redacted] induces skin sensitization in mice after three epidermal exposures of the animals under the conditions described in this study plan. This study should provide a rational basis for risk assessment in man and data produced can be used for classification/labelling of the test item. Compared to sensitization tests using guinea pigs, the Local Lymph Node Assay (LLNA) provides certain advantages with regard to animal welfare and scientific aspects.

The design of this study is in compliance with the following study guidelines:

- OECD Guideline 429. *Skin Sensitization: Local Lymph Node Assay,* July 2010.
- EC No 640/2012 Part B. *Skin Sensitization: Local Lymph Node Assay,* July 2012.
- EPA Health Effects Test Guideline OPPTS 870.2600. *Skin Sensitization,* March 2003.

The Study Director signed the study plan on 22 Jul 2019, and dosing was initiated on 31 Jul 2019. The in-life phase of the study was completed on 19 Aug 2019. The experimental start date was 26 Jul 2019, and the experimental completion date was 20 Aug 2019. The study plan and deviations are presented in Appendix 4.

4. MATERIALS AND METHODS

4.1. Test item and Vehicle

4.1.1. Test Item

Identification:	[redacted]
Batch (Lot) Number:	2
Expiry date:	15 April 2020 (expiry date)
Physical Description:	Clear colourless very highly viscous liquid
Purity/Composition:	>99%
Storage Conditions:	At room temperature
Additional information	
Test Facility test item number:	[redacted]
Stability at higher temperatures:	Maximum temperature: <71°C Maximum duration: Only as long as necessary; the test article should not deteriorate over the period of time it takes to warm it up to temperature. Not for longer than necessary since there is no data on stability at this temperature.
Chemical name (IUPAC, synonym or trade name):	Urethane Resin; [redacted]
Molecular weight:	[redacted]
General information:	The test article is a complex reaction product. >99% of the test article is the desired complex reaction product. [redacted]

4.1.2. Vehicle

N,N-dimethylformamide (DMF) (Merck, Darmstadt, Germany).

4.1.2.1. Rationale for Vehicle

The vehicle was chosen from the vehicles specified in the test guideline (in order of preference): Acetone/Olive oil (4:1 v/v), N,N-dimethylformamide, methylethylketone, propylene glycol, dimethylsulfoxide and 1% Pluronic© L92 in Elix water (in case an aqueous vehicle is suitable). The vehicle was selected on the basis of maximizing the solubility based on trial preparations performed at Charles River Den Bosch and on information provided by the Sponsor. Trial preparations were performed to select the suitable vehicle and to establish a suitable formulation procedure. These trials were not performed as part of this study and these preparations were not used for dosing. Raw Data of these trials will be retained by the Test Facility. There was no information available about the stability and solubility of the test item in vehicle.

4.2. Test Item Characterization

The Sponsor provided to the Test Facility documentation of the identity, purity, composition, and stability for the test item. The characterization of the test item was conducted in a GLP quality environment. A Certificate of Analysis was provided to the Test Facility and is presented in Appendix 2.

4.3. Reserve Samples

For each batch (lot) of test item, a reserve sample (about 0.5 gram) was collected and maintained under the appropriate storage conditions by the Test Facility. The sample will be destroyed after the expiry date.

4.4. Test and Reference Item Inventory and Disposition

Records of the receipt, distribution, and storage of test item were maintained. With the exception of reserve samples, all unused Sponsor-supplied test item will be discarded. Records of the decisions made will be kept at the Test Facility.

4.5. Preparation of Test Item

Test item dosing formulations (w/w) were homogenized to visually acceptable levels by heating up in a water bath set at 60 degrees °C for approximately 15 minutes. The formulations were allowed to cool down to room temperature prior to dosing. The sponsor indicated that no decomposition/deterioration would be expected to occur under these conditions.

The dosing formulations were prepared daily and dosed within 4 hours after adding the vehicle to the test item.

The dosing formulations were kept at room temperature until dosing. The dosing formulations were stirred until and during dosing.

No adjustment was made for specific gravity of the vehicle and no correction was made for the purity/composition of the test item, since the test method requires a logical concentration range rather than specific dose levels.

Any residual volumes were discarded.

4.6. Sample Collection and Analysis

Analysis of test item in vehicle for concentration, stability, homogeneity was not performed.

4.7. Test System

Species:	Mouse
Strain:	CBA/J
Condition:	Inbred, SPF-Quality
Source:	Janvier, Le Genest-Saint-Isle, France
Number of Animals:	20 Females (nulliparous and non-pregnant). Five females per group.
Age at the Initiation of Dosing:	Young adult animals (approximately 10 weeks old) were selected.
Weight at the Initiation of Dosing:	16.7 to 23.2 g.

4.7.1. Justification for Test System and Number of Animals

The CBA/J mouse was chosen as the animal model for this study as recognized by international guidelines as a recommended test system (e.g. OECD, FDA, MHLW). The test method and number of animals were based on the test guidelines.

The results of a reliability test with three concentrations of Hexylcinnamaldehyde (CAS No. 101-86-0) in Acetone/Olive oil (4:1 v/v), performed not more than 6 months previously and using the same materials, animal supplier, animal strain and essential procedures are summarized in Appendix 3 of this report. For both scientific and animal welfare reasons, no concurrent positive control group was included in the study. An extensive data base is available with reliability checks performed each half year during at least the recent 9 years showing reproducible and consistent positive results.

The study plan was reviewed and agreed by the Animal Welfare Body of Charles River Laboratories Den Bosch B.V. within the framework of Appendix 1 of project license AVD2360020172866 approved by the Central Authority for Scientific Procedures on Animals (CCD) as required by the Dutch Act on Animal Experimentation (December 2014).

4.7.2. Animal Identification

At study assignment, each animal was identified using a tail mark with indelible ink.

4.7.3. Environmental Acclimation

The animals were allowed to acclimate to the Test Facility toxicology accommodation for at least 5 days before the commencement of dosing.

4.7.4. Selection, Assignment, Replacement, and Disposition of Animals

Animals were assigned to the study at the discretion of the coordinating biotechnician, with all animals within ± 20% of the sex mean body weights. Animals in poor health or at extremes of body weight range were not assigned to the study.

Before the initiation of dosing, a health inspection was performed and any assigned animal considered unsuitable for use in the study were replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

The disposition of all animals was documented in the study records.

4.7.5. Husbandry

4.7.5.1. Housing

On arrival and following assignment to the study, animals were group housed (up to 5 animals of the same sex and same dosing group together) in polycarbonate cages (Makrolon MIII type; height 18 cm.) containing sterilized sawdust as bedding material (Lignocel S 8-15, JRS - J.Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany) equipped with water bottles. The rooms in which the animals were kept were documented in the study records.

Animals were separated during designated procedures/activities. Each cage was clearly labeled.

4.7.5.2. Environmental Conditions

Target temperatures of 18 to 24°C with a relative target humidity of 40 to 70% were maintained. The actual daily mean temperature during the study period was 22 to 23°C with an actual daily mean relative humidity of 52 to 79%. The values that were outside the targeted range occurred for three days with a maximum of 79% and were without a noticeable effect on the clinical condition of the animals or on the outcome of the study. A 12-hour light/12-hour dark cycle was maintained. Ten or greater air changes per hour with 100% fresh air (no air recirculation) were maintained in the animal rooms.

4.7.5.3. Food

Pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany) was provided ad libitum throughout the study, except during designated procedures.

The feed was analyzed by the supplier for nutritional components and environmental contaminants. Results of the analysis were provided by the supplier and are on file at the Test Facility.

It is considered that there were no known contaminants in the feed that would interfere with the objectives of the study.

4.7.5.4. Water

Municipal tap-water was freely available to each animal via water bottles.

Periodic analysis of the water was performed, and results of these analyses are on file at the Test Facility.

It is considered that there were no known contaminants in the water that would interfere with the objectives of the study.

4.7.5.5. Animal Enrichment

For psychological/environmental enrichment, animals were provided with paper (Enviro-dri, Wm. Lillico & Son (Wonham Mill Ltd), Surrey, United Kingdom) and shelters (disposable paper corner home, MCORN 404, Datesand Ltd, USA), except when interrupted by study procedures/activities.

4.7.5.6. Veterinary Care

Veterinary care was available throughout the course of the study; however, no examinations or treatments were required.

4.8. Experimental Design

4.8.1. Justification of Route and Dose Levels

Dose route and dose concentrations used are in compliance with the OECD test guidelines for LLNA studies.

4.8.2. Pre-screen Test

A pre-screen test was conducted in order to select the highest test item concentration to be used in the main study. In principle, this highest concentration should cause no systemic toxicity, may give well-defined irritation as the most pronounced response (maximum grade 2 and/or an increase in ear thickness < 25%) and/or is the highest possible concentration that can technically be applied.

Two test item concentrations were tested; a 25% and 50% concentration. The highest concentration was the highest concentration that could be prepared homogeneously.

The test system, procedures and techniques were identical to those used in the main study except that the animals were approximately 11 weeks (at initiation of treatment) and that the assessment of lymph node proliferation and necropsy were not performed. Two young adult females per concentration were selected. Each animal was treated with one concentration on three consecutive days. Animals were group housed in labeled Makrolon cages (MII type, height 14 cm). Ear thickness measurements were conducted using a digital thickness gauge (Kroeplin C110T-K) prior to dosing on Days 1 and 3, and on Day 6.

Animals were sacrificed after the final observation.

4.8.3. Main Study

Three groups of five animals were treated with one test item concentration per group. The highest test item concentration was selected from the pre-screen test. One group of five animals was treated with the vehicle.

4.8.3.1. Allocation

Text table 1
Allocation

Group[1]	animal numbers		induction (test item; % w/w)
1	01 - 05	Vehicle control	0 (N,N-dimethylformamide (DMF))
2	06 - 10	Experimental low concentration	10
3	11 - 15	Experimental Intermediate concentration	25
4	16 - 20	Experimental high concentration	40

[1] five females per group

4.8.3.2. Induction - Days 1, 2 and 3

The dorsal surface of both ears was topically treated (25 µL/ear) with the test item, at approximately the same time on each day. The concentrations were stirred with a magnetic stirrer immediately prior to dosing.

The control animals were treated in the same way as the experimental animals, except that the vehicle was administered instead of the test item.

4.8.3.3. Excision of the Nodes - Day 6

Each animal was injected via the tail vein with 0.25 mL of sterile phosphate buffered saline (PBS) (Merck, Darmstadt, Germany) containing 20 µCi of ^{3}H-methyl thymidine (PerkinElmer Life and Analytical Sciences, Boston, MA, US).

After five hours, all animals were euthanized by intraperitoneal injection (0.2 mL/animal) of Euthasol® 20% (AST Farma BV, Oudewater, The Netherlands). The draining (auricular) lymph node of each ear was excised. The relative size of the nodes (as compared to normal) was estimated by visual examination and abnormalities of the nodes and surrounding area were recorded. The nodes were pooled for each animal in PBS.

4.8.3.4. Tissue Processing for Radioactivity - Day 6

Following excision of the nodes, a single cell suspension of lymph node cells (LNC) was prepared in PBS by gentle separation through stainless steel gauze (maze size: 200 µm, diameter: ± 1.5 cm). LNC were washed twice with an excess of PBS by centrifugation at 200*g* for 10 minutes at 4°C. To precipitate the DNA, the LNC were exposed to 5% trichloroacetic acid (TCA) (Merck, Darmstadt, Germany) and then stored in the refrigerator until the next day.

4.8.3.5. Radioactivity Measurements - Day 7

Precipitates were recovered by centrifugation, resuspended in 1 mL TCA and transferred to 10 mL of Ultima Gold cocktail (PerkinElmer Life and Analytical Sciences, Boston, MA, US) as the scintillation fluid. Radioactivity measurements were performed using a Packard scintillation counter (2910TR). Counting time was to a statistical precision of ± 0.2% or a maximum of 5 minutes whichever came first. The scintillation counter was programmed to automatically subtract background and convert Counts Per Minute (CPM) to Disintegrations Per Minute (DPM).

4.9. In-life Procedures, Observations, and Measurements

4.9.1. Mortality/Moribundity Checks

Throughout the study, animals were observed for general health/mortality and moribundity twice daily, in the morning and at the end of the working day. Animals were not removed from cage during observation, unless necessary for identification or confirmation of possible findings.

4.9.2. Clinical Observations

4.9.2.1. Postdose Observations

Postdose observations were performed once daily on Days 1-6 (on Days 1-3 between 3 and 4 hours after dosing).

All the animals were examined for reaction to dosing. The onset, intensity and duration of these signs was recorded (if appropriate), particular attention being paid to the animals during and for the first hour after dosing.

4.9.3. Body Weights

Animals were weighed individually on Day 1 (predose) and 6 (prior to necropsy).

4.9.4. Irritation

Erythema and eschar formation observations were performed once daily on Days 1-6 (on Days 1-3 within 1 hour after dosing), according to the following numerical scoring system. Furthermore, a description of all other (local) effects was recorded.

Erythema and eschar formation:

No erythema 0
Very slight erythema (barely perceptible) 1
Well-defined erythema 2
Moderate to severe erythema (beet redness) to slight eschar formation (injuries in depth) 3
Severe erythema (beet redness) to eschar formation preventing grading of erythema 4

4.10. Terminal procedures

No necropsy was performed, since all animals survived until the end of the observation period.

5. ANALYSIS

All results presented in the tables of the report are calculated using values as per the raw data rounding procedure and may not be exactly reproduced from the individual data presented.

DPM values are presented for each animal and for each dose group. A Stimulation Index (SI) is calculated for each group using the individual SI values. The individual SI is the ratio of the DPM/animal compared to the DPM/vehicle control group mean.

If the results indicate a SI ≥ 3, the test item may be regarded as a skin sensitizer.

The results were evaluated according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2017) (including all amendments) and the Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of items and mixtures, including all amendments.

Consideration was given to the EC3 value (the estimated test item concentration that will give a SI =3) (reference 1).

Text Table 2
Classification of results

	UN-GHS 2017; EC-CLP 2008	EC Hazard statement
SI < 3	No sensitizer	
SI ≥ 3	Cat 1 Skin sensitizer EC3 value ≤ 2%: sub-category 1A EC3 value > 2%: sub-category 1B	H317: May cause an allergic skin reaction

6. COMPUTERIZED SYSTEMS

Critical computerized systems used in the study are listed below. All computerized systems used in the conduct of this study have been validated; when a particular system has not satisfied all requirements, appropriate administrative and procedural controls were implemented to assure the quality and integrity of data.

Text Table 3
Critical Computerized Systems

System name	Version No.	Description of Data Collected and/or Analyzed
Deviation Information Library	2.1.68	Deviations
REES Centron	SQL 2.0	Temperature, relative humidity and/or atmospheric pressure monitoring Animal and Laboratory facilities
Quantasmart	4.01	Scintillation counts System control and data acquisition

7. RETENTION OF RECORDS

All study-specific raw data, documentation, study plan and final report from this study were archived at the Test Facility by no later than the date of final report issue. At least two years after issue of the final report, the Sponsor will be contacted.

Electronic data generated by the Test Facility were archived as noted above, except that files stored on SDMS (Study Plan (amendments) and reporting files) and study deviations were archived at the Charles River Laboratories facility location in Wilmington, Massachusetts, USA.

8. RESULTS

For detailed results see Appendix 1.

8.1. Pre-screen Test

At a 25% and 50% test item concentration, no signs of systemic toxicity were noted and up to very slight irritation were observed and therefore the 50% concentration was selected as highest concentration for the main study. Inadvertently however, the main study was performed with 40% concentration due to a logistical error. This did not affect the outcome of the study (see deviations Appendix 4).

8.2. Main Study

8.2.1. Skin Reactions / Irritation

Very slight erythema and scaliness of the ears was seen for the animals dosed at 25% and 40%. Scabs on the ears were noted for one animal dosed at 25% and one animal dosed at 40%. The findings were considered not to have a toxicologically significant effect on the activity of the nodes.

White test item remnants were present on the dorsal surface of the ears of all animals at 40% and some animals at 25%, which did not hamper scoring of the skin reactions. Bald skin spots behind the ears was noted for the majority of the animals treated at 40%.

8.2.2. Systemic Toxicity

No mortality occurred and no clinical signs of systemic toxicity were observed in the animals. Body weights and body weight gain of experimental animals remained in the same range as controls over the study period.

8.2.3. Macroscopic Examination of the Lymph Nodes and Surrounding Area

All auricular lymph nodes of the test item treated animals were enlarged, compared to the controls. The largest auricular lymph nodes were found in the higher dose groups. No macroscopic abnormalities of the surrounding area were noted for any of the animals.

8.2.4. Radioactivity Measurements and SI Values

Mean DPM/animal values for the experimental groups treated with test item concentrations 10, 25 and 40% were 4838, 8691 and 18271 DPM, respectively. The mean DPM/animal value for the vehicle control group was 602 DPM. The SI values calculated for the test item concentrations 10, 25 and 40% were 8.0, 14.4 and 30.4, respectively.

9. CONCLUSION

These results show that the test item elicits a SI ≥ 3. The EC3 value (the estimated test item concentration that will give a SI =3) was established to be between >0 and 10%. No reliable EC3 value could be calculated by the method of Ryan et al. (2007) because the lowest SI value (SI of 8.0) does not approach the SI=3 value.

The six-month reliability check with Alpha-hexylcinnamaldehyde indicates that the Local Lymph Node Assay as performed at Charles River Den Bosch is an appropriate model for testing for contact hypersensitivity (see Appendix 3).

Based on these results:

- According to the recommendations made in the test guidelines (including all amendments), [redacted] would be regarded as skin sensitizer.
- According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2017) (including all amendments), [redacted] should be classified as skin sensitizer (Category 1).
- According to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of items and mixtures (including all amendments), [redacted] should be classified as skin sensitizer (Category 1) and labeled as H317: May cause an allergic skin reaction.

10. REFERENCES

1 Basketter DA, Lea LJ, Dickens A, Briggs, D, Pate I, Dearman RJ and Kimber I. A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses. J Appl Toxicol 1999;19:261-266.

2 Ryan et al, Extrapolating local lymph node assay EC3 values to estimate relative sensitizing potency. Cutaneous and Ocular Toxicology, 26: 135–145, 2007

Appendix 1
Tables and Figures

Table 1 Pre-Screen Test: Body Weights and Skin Reactions

TS [1] (%)	animal	Day 1 bw (g) [2]	Day 1 erythema[3] left	Day 1 erythema[3] right	Day 2 erythema left	Day 2 erythema right	Day 3 erythema left	Day 3 erythema right	Day 4 erythema left	Day 4 erythema right	Day 5 erythema left	Day 5 erythema right	Day 6 erythema left	Day 6 erythema right	Day 6 bw (g)
25	1	25.0	0	0	0	0	1	1	1	1	0	0	0	0	25.1
	2	25.0	0	0	0	0	1	1	1	1	0	0	0	0	24.1
50	3	20.9	0	0	0f	0f	1ft	1ft	1ft	1ft	1t	1t	1t	1t	21.0
	4	23.0	0	0	0f	0f	1ft	1ft	1ft	1ft	1t	1t	1t	1t	22.1

f. White staining of the dorsal surface of the ears by test item remnants which did not hamper the scoring of the ears. t. Bald skin spots behind the ears.

[1] TS = test item (% w/w).

[2] Body weight (grams).

[3] Grading erythema and eschar formation (Left = dorsal surface of left ear; right = dorsal surface of right ear):
0 = No erythema
1 = Very slight erythema (barely perceptible)

Table 2 Pre-Screen Test: Ear Thickness Measurements

TS [1] (%)	Animal	Day 1 left (mm)	Day 1 right (mm)	Day 3 left (mm)	Day 3 left % [2]	Day 3 right (mm)	Day 3 right % [2]	Day 6 left (mm)	Day 6 left % [2]	Day 6 right (mm)	Day 6 right % [2]
25	1	0.215	0.220	0.235	9	0.240	9	0.250	16	0.255	16
	2	0.225	0.230	0.245	9	0.245	7	0.250	11	0.250	9
50	3	0.230	0.230	0.250	9	0.250	9	0.270	17	0.270	17
	4	0.235	0.230	0.255	9	0.250	9	0.275	17	0.270	17

Left (mm) = thickness of left ear in millimeters; right (mm) = thickness of right ear in millimeters.

[1] TS = test item (% w/w).

[2] Percent increase compared to Day 1 pre-dose value. A 25% value is used as the threshold for selection for use in the main study.

Table 3 Main Study: Body Weights and Skin Reactions

group	TS [1] (%)	animal	Day 1			Day 2		Day 3		Day 4		Day 5		Day 6		
			bw (g) [2]	erythema [3] left	right	erythema left	right	erythema left	right	erythema left	right	erythema left	right	erythema left	right	bw (g)
1	0	1	18.9	0	0	0	0	0	0	0	0	0	0	0	0	18.3
		2	19.3	0	0	0	0	0	0	0	0	0	0	0	0	20.9
		3	21.3	0	0	0	0	0	0	0	0	0	0	0	0	22.1
		4	21.4	0	0	0	0	0	0	0	0	0	0	0	0	23.1
		5	20.0	0	0	0	0	0	0	0	0	0	0	0	0	18.9
2	10	6	21.8	0	0	0	0	0	0	0	0	0	0	0	0	23.1
		7	20.4	0	0	0	0	0	0	0	0	0	0	0	0	23.2
		8	20.1	0	0	0	0	0	0	0	0	0	0	0	0	21.9
		9	20.2	0	0	0	0	0	0	0	0	0	0	0	0	24.6
		10	23.2	0	0	0	0	0	0	0	0	0	0	0	0	23.3
3	25	11	21.0	0	0	1	1	1	1	1	1	1	1	1	1	23.1
		12	16.7	0	0	0	0	0	0	1s	1	1s	1s	1s	1s	15.4
		13	21.7	0	0	0f	0f	1f	1f	1s	1k	1s	1k	1s	1s	22.6
		14	19.9	0	0	0	0	0	0	1	1	1	1	1	1	22.0
		15	22.3	0	0	1	0f	1f	1f	1	1	1	1	1	1	24.4
4	40	16	20.8	1f	0f	1f	1f	1f	1ft	1ks	1fst	1ks	1ks	1s	1st	23.1
		17	19.9	1f	1f	1f	1f	1f	1ft	1s	1ft	1s	1s	1s	1st	20.4
		18	21.5	0f	1f	1f	1f	1f	1ft	1s	1st	1s	1s	1s	1st	23.3
		19	20.9	1f	1f	1f	1f	1f	1ft	1f	1st	1s	1s	1	1	23.3
		20	21.7	1f	1f	1f	1f	1f	1ft	1f	1ft	1s	1s	1s	1st	24.4

f. White staining of the dorsal surface of the ears by test item remnants which did not hamper the scoring of the ears, k. Scabs, s. Scaliness, t. Bald skin spots behind the ears.

[1] TS = test item (% w/w).

[2] Body weight (grams).

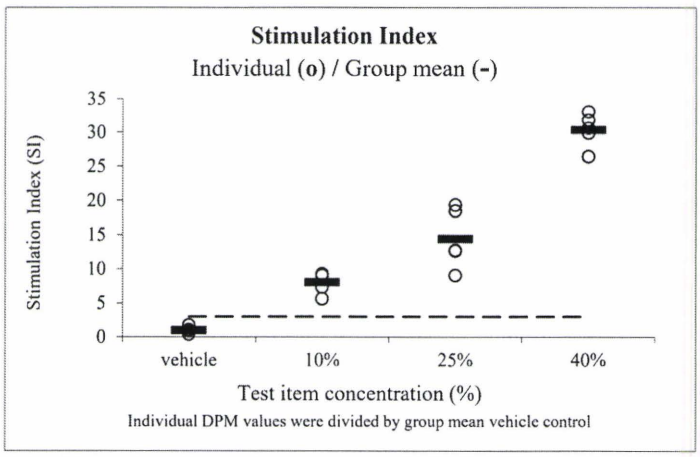
[3] Grading erythema and eschar formation (Left = dorsal surface of left ear; right = dorsal surface of right ear):
0 = No erythema
1 = Very slight erythema (barely perceptible)

Table 4 Main Study: Relative Size Lymph Nodes, Radioactivity Counts (DPM) and Stimulation Index (SI)

group	TS [1] (%)	animal	Size nodes [2] left	Size nodes [2] right	DPM [3] / animal	mean DPM ± SEM [4]	mean SI ± SEM
1	0	1	n	n	242		
		2	n	n	569		
		3	n	n	1052	602 ± 131	1.0 ± 0.2
		4	n	n	503		
		5	n	n	643		
2	10	6	+	+	3381		
		7	+	+	5427		
		8	+	+	4381	4838 ± 422	8.0 ± 0.7
		9	+	+	5449		
		10	+	+	5551		
3	25	11	+	+	11117		
		12	+	+	5426		
		13	+	+	7656	8691 ± 1174	14.4 ± 2.0
		14	+	+	7605		
		15	+	+	11650		
4	40	16	++	++	17988		
		17	++	++	18421		
		18	++	++	15896	18271 ± 677	30.4 ± 1.1
		19	++	++	19895		
		20	++	++	19153		

[1] TS = test item (% w/w).
[2] Relative size auricular lymph nodes (-, -- or ---: degree of reduction, +, ++ or +++: degree of enlargement, n: considered to be normal).
[3] DPM = Disintegrations per minute.
[4] SEM = Standard Error of the Mean.

Figure 1. Dose-Response Curve



Appendix 2
Test Item Characterization

[redacted]

Product Test	QC Comment	UOM	Lower Limit	Target	Upper Limit	Ind Result	Test Method
Isocyanate Content		%			0.001	0.004	50.7.194
Viscosity Rheometer		cP				9317	41.24.13
Appearance	No bubbles present			PASS		FAIL	22.74.C

[redacted]

Appendix 3
Reliability check

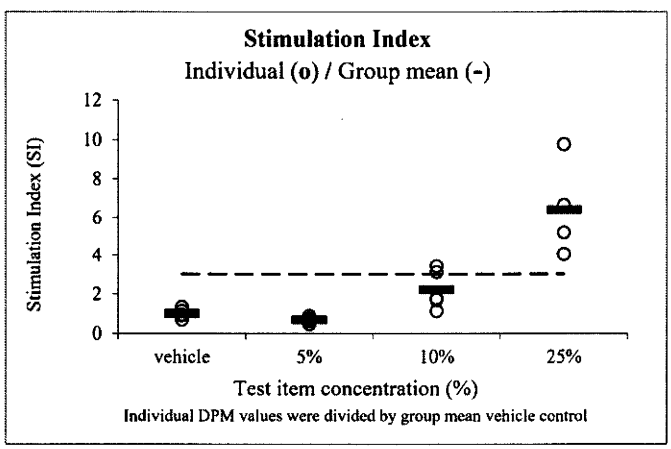
ASSESSMENT OF CONTACT HYPERSENSITIVITY
TO ALPHA- HEXYLCINNAMALDEHYDE, TECHNICAL GRADE
IN THE MOUSE (LOCAL LYMPH NODE ASSAY)
A RELIABILITY CHECK
Test Facility Study No. [redacted]

SUMMARY RELIABILITY CHECK

A reliability check is carried out at regular intervals to check the sensitivity of the test system and the reliability of the experimental techniques as used by Charles River Den Bosch. In this study, performed in July 2019, females of the CBA/J mouse strain (Janvier, Le Genest-Saint-Isle, France) were checked for sensitivity to Alpha- Hexylcinnamaldehyde, technical grade (HCA). The females were approximately 10 weeks old at commencement of the study. The study was based on the OECD Guideline No. 429, EC No 440/2008, Part B.42 and EPA, OPPTS 870.2600 "Skin Sensitization". Alpha- Hexylcinnamaldehyde, technical grade (CAS no. 101-86-0) was fabricated under lot no. MKCD3159 (Sigma- Aldrich, Steinheim, Germany). Concentrations used for this study were 5, 10 and 25% in Acetone/Olive oil (4:1 v/v; AcOO).

Group[1]	% HCA	mean DPM ± SEM	SI ± SEM
1	0% (AcOO)	747 ± 80	1.0 ± 0.1
2	5%	516 ± 52	0.7 ± 0.1
3	10%	1663 ± 330	2.2 ± 0.4
4	25%	4786 ± 926	6.4 ± 1.2

[1] Five females per group, for Group 4 nodes of one animal are missing due to a technical error.



CONCLUSION

The SI values calculated for the item concentrations 5, 10 and 25% were 0.7, 2.2 and 6.4 respectively. An EC3 value of 12.8% was calculated using linear interpolation.

The calculated EC3 value was found to be in the acceptable range of 4.8 and 19.5%. The results of the 6 monthly HCA reliability checks of the recent years were 14.1, 17.3, 9.8, 17.8, 14.3 and 16.3%.

Based on these results it was concluded that the Local Lymph Node Assay as performed at Charles River Den Bosch is an appropriate model for testing for contact hypersensitivity.

The raw data, study plan and report from this study are kept in the Charles River Den Bosch archives. The test described above was performed in accordance with Charles River Den Bosch Standard Operating Procedures and the report was audited by the QA-unit.

Appendix 4
Study Plan and Deviations

charles river

FINAL STUDY PLAN

Test Facility Study No. [redacted]

Sponsor Reference No. [redacted]

Assessment of Skin Sensitization to [redacted] in the Mouse (Local Lymph Node Assay)

SPONSOR:

[redacted]

TEST FACILITY:
Charles River Laboratories Den Bosch B.V.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

TABLE OF CONTENTS

1. OBJECTIVE(S)

The objective of this study is to evaluate whether [redacted] induces skin sensitization in mice after three epidermal exposures of the animals under the conditions described in this study plan. This study should provide a rational basis for risk assessment in man and data produced can be used for classification/labelling of the test item. Compared to sensitization tests using guinea pigs, the Local Lymph Node Assay (LLNA) provides certain advantages with regard to animal welfare and scientific aspects.

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual dates will be included in the Final Report.

Experimental Starting Date:	25 Jul 2019 (Week 30) (First date of study-specific data collection)
Experimental Completion Date:	22 Sep 2019 (Week 38) (Last date on which data are collected)
Initiation of Dosing:	29 Jul 2019 (Week 31)
Completion of In-life:	15 Sep 2019 (Week 37) (Last date of necropsy)
Unaudited Draft Report:	29 Sep 2019 (Week 39)

3. SPONSOR

[redacted]

4. RESPONSIBLE PERSONNEL

Role/Phase	Quality Assurance Unit	Name	Contact Information
Study Director	Charles River	A.H.B.M. van Huygevoort, MSc	Address as cited for Test Facility Tel: +31 73 640 6700 E-mail: Pieter.vanSas@crl.com
Test Facility Management	Charles River	H.H. Emmen, MSc	Address as cited for Test Facility Tel: +31 73 640 6700 E-mail: harry.emmen@crl.com
Test Facility QAU	Charles River	C.J. Mitchell, BSc	Address as cited for Test Facility Tel: +31 73 640 6700 E-mail: QADenBosch@crl.com

5. TEST MATERIALS

5.1. Test Item Characterization

The Sponsor will provide to the Test Facility documentation of the identity, strength, purity, composition, and stability for the test item. A Certificate of Analysis or equivalent documentation may be provided for inclusion in the Final Report.

5.2. Test Material Identification

5.2.1. Test Item

Identification:	[redacted]
Batch (Lot) Number:	2
Expiry date:	15 April 2020 (expiry date)
Physical Description:	Clear colourless very highly viscous liquid
Purity/Composition:	>99%
Storage Conditions:	At room temperature

Additional information

Test Facility test item number:	210429/A
Stability at higher temperatures:	Maximum temperature: <71°C
Chemical name (IUPAC, synonym or trade name):	Urethane Resin; [redacted]
Molecular weight:	[redacted]
General information:	The test article is a complex reaction product. >99% of the test article is the desired complex reaction product. [redacted]

5.2.2. Vehicle

N,N-dimethylformamide (DMF).

5.3. Reserve Samples

For each batch (lot) of test item and if practically possible, a reserve sample will be collected and maintained under the appropriate storage conditions by the Test Facility.

5.4. Test Item Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of test materials will be maintained.

5.5. Safety

The following safety instruction(s) apply to this study:

- Standard safety precautions specified in Charles River Den Bosch procedures

6. DOSE FORMULATION AND ANALYSIS

6.1. Preparation of Formulations

Preparation Details

Dose Formulation	Procedure	Frequency of Preparation	Storage Conditions
Vehicle	- used as available - stirred until dosing	Not applicable	Kept at room temperature until use
Test Item	- prepared on w/w basis - stirred until dosing - no correction for purity/composition of the test item[a] - no adjustment for specific gravity of the test item and vehicle[a] - in order to obtain homogeneity to visually acceptable levels, heat up formulations in a water bath set at 60 degrees C for approximately 15 minutes - allow to cool down to room temperature prior to dosing	Daily, dosed within 4 hours after adding the vehicle	Kept at room temperature until use

[a] the test method requires a logical concentration range rather than specific dose levels

Any residual volumes from each dosing occasion will be discarded unless otherwise requested by the Study Director.

6.2. Trial Preparations

Trial preparations were performed to select the suitable vehicle and to establish a suitable formulation procedure. These trials were not performed as part of this study were not used for dosing. Raw Data of these trials will be retained by the Test Facility.

6.3. Sample Collection and Analysis

Analysis of test item in vehicle for concentration, stability, homogeneity will not be performed, however, to limit the impact, the test item preparation will be performed with approved procedures and documented in detail. Formulations will be visually inspected for homogeneity prior to use and all formulations will be used within 4 hours after adding vehicle to the test item. This GLP exception was therefore considered as being minor with no impact on the outcomes and the integrity and the achievement of the objective of the study.

7. TEST SYSTEM

Species:	Mice
Strain:	CBA/J
Condition:	Inbred, SPF-Quality

Source:	Based on availability, one of the following sources will be used and specified in the report: • Charles River France, L'Arbresle, France • Charles River Deutschland, Sulzfeld, Germany • Janvier, Le Genest-Saint-Isle, France
Number of Animals:	20 females (nulliparous and non-pregnant), 5 females per group.
Target Age at the Initiation of Dosing:	Between 8 and 12 weeks old. Animals to be used within the study will be of approximately the same age.
Target Weight at the Initiation of Dosing:	15 to 25 g.

The actual age and weight of the animals at the initiation of dosing will be listed in the Final Report.

7.1. Animal Identification

Method: Each animal will be identified using a tail mark with indelible ink. Further identification marks may be applicable, to be documented in the study file.

7.2. Environmental Acclimation

The animals will be allowed to acclimate to the Test Facility toxicology accommodation for at least 5 days before the commencement of dosing.

7.3. Selection, Assignment, Replacement, and Disposition of Animals

Selection: Animals will be assigned to the study at the discretion of the coordinating biotechnician according to body weights, with all animals within ± 20% of the sex mean. Animals in poor health or at extremes of body weight range will not be assigned to the study.

Replacement: Before the initiation of dosing, any assigned animals considered unsuitable for use in the study will be replaced by alternate animals obtained from the same shipment and maintained under the same environmental conditions.

Disposition: The disposition of all animals will be documented in the Study Files.

8. HUSBANDRY

8.1. Housing

Caging: Group housed (up to 5 animals of the same sex and same dosing group together) in polycarbonate cages (Makrolon MIII type; height 18 cm.) containing sterilized sawdust as bedding material (Lignocel S 8-15, JRS - J.Rettenmaier & Söhne GmbH + CO. KG, Rosenberg, Germany)

	equipped with water bottles. These housing conditions will be maintained unless deemed inappropriate by the Study Director and/or Clinical Veterinarian. The room(s) in which the animals will be kept will be documented in the study records.
Cage Identification:	Cage cards indicating at least Test Facility Study No., group, animal number(s).

8.2. Animal Enrichment

For psychological/environmental enrichment, animals will be provided with paper (Enviro-dri, Wm. Lillico & Son (Wonham Mill Ltd), Surrey, United Kingdom) and shelters (disposable paper corner home, MCORN 404, Datesand Ltd, USA), except when interrupted by study procedures/activities.

8.3. Environmental Conditions

The targeted conditions for animal room environment will be as follows:

Temperature:	18 to 24°C
Humidity:	40 to 70%
Light Cycle:	12 hours light and 12 hours dark (except during designated procedures)
Ventilation:	Ten or more air changes per hour

8.4. Food

Diet:	SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany
Type:	Pellets (alternate diet may be provided on individual animal basis as warranted as approved by the Study Director).
Frequency:	Ad libitum, except during designated procedures.
Analysis:	Results of analysis for nutritional components and environmental contaminants are provided by the supplier and are on file at the Test Facility. It is considered that there are no known contaminants in the feed that would interfere with the objectives of the study.

8.5. Water

Type:	Municipal tap water.
Frequency/Ration:	Freely available to each animal via water bottles.
Analysis:	Periodic analysis of the water is performed, and results of these analyses are on file at the Test Facility. It is considered that there are no known contaminants in the water that could interfere with the outcome of the study.

8.6. Veterinary Care

Veterinary care will be available throughout the course of the study and animals will be examined by the veterinary staff as warranted by clinical signs or other changes. In the event that animals show signs of illness or distress, the responsible veterinarian may make initial recommendations about treatment of the animal(s) and/or alteration of study procedures, which must be approved by the Study Director. Treatment of the animal(s) for minor injuries or ailments may be approved without prior consultation with the Sponsor representative when such treatment does not impact fulfillment of the study objectives. If the condition of the animal(s) warrants significant therapeutic intervention or alterations in study procedures, the Sponsor representative will be contacted, when possible, to discuss appropriate action. If the condition of the animal(s) is such that emergency measures must be taken, the Study Director and/or attending veterinarian will attempt to consult with the Sponsor representative prior to responding to the medical crisis, but the Study Director and/or veterinarian has authority to act immediately at his/her discretion to alleviate suffering. The Sponsor representative will be fully informed of any such events.

9. EXPERIMENTAL DESIGN

9.1. Pre-screen Test

A pre-screen study will be conducted after approval by the Study Director in the study files, in order to select the highest test item concentration to be used in the main study. In principle, this highest concentration should cause no systemic toxicity, may give well-defined irritation as the most pronounced response (maximum erythema Grade 2 and/or an increase in ear thickness < 25%) and/or is the highest possible concentration that can technically be applied. However, the selection may depend on a number of other factors and exact criteria do not always apply.

Four young adult animals (females, 8 -12 weeks old) will be selected and two concentrations will be tested, each on two animals. In principle, the concentrations will be selected from the series 100% (undiluted), 50%, 25%, 10%, 5%, 2%, 1% (or lower concentrations using the same steps), taking toxicity data, item properties and technical feasibility into account. Intermediate concentrations may be selected based on trial formulation results and approved by the Study Director in the study files. Additional animals may be used following approval by the Study Director in the study files if results do not meet the selection criteria.

The procedures and techniques will be the same as those used in the main study, with the exceptions that the assessment of lymph node proliferation and necropsy will not be

performed. Animals will be group housed in labeled Makrolon cages (MII type, height 14 cm) and ear thickness measurements will be conducted using a digital thickness gauge prior to dosing on Days 1 and 3, and on Day 6. Animals will be sacrificed after the final observation.

If test item remnants interfere with scoring for erythema or if the ear thickness measurements may be influenced by these remnants, the ears may be cleaned using tap water and/or the selected vehicle on Days 2, 3 and/or 6 and scoring for erythema on these days will only be done following the sequence of events indicated below:

Day 2.: clean if still needed → score erythema (if cleaned score not within 30 min.) → dosing

Day 3.: clean if needed → score erythema (if cleaned score not within 30 min.) → ear thickness and dosing

Day 6.: clean if still needed → score erythema (if cleaned score not within 30 min.) → ear thickness

9.2. Main Study

Three test item concentrations will be used in the main study, selected and approved by the Study Director in the study files. The concentrations will be taken from the series.: 100% (undiluted), 50%, 25%, 10%, 5%, 2%, 1% and lower concentrations using the same steps). If needed, intermediate concentrations may be selected. A vehicle control group will be added in the main study.

9.2.1. Allocation

Group No.	Group Id.	Dose Volume (uL per ear)	Dose Concentration (%)	Number of Females	Animal Numbers
1	Vehicle Control	25	0 (Vehicle)	5	1-5
2	Experimental	25	Low	5	6-10
3	Experimental	25	Intermediate	5	11-15
4	Experimental	25	High	5	16-20

9.3. Administration of Test Materials

Dose Route: Epidermal

Frequency: Once daily

Duration: Days 1 to 3

Method: The first day of dosing will be designated as Day 1. The dose formulations will be stirred continuously during dosing, if practically possible. The doses will be applied on the dorsal surface of the ears (25 µL/ear) approximately the same time each day.

10. IN-LIFE PROCEDURES, OBSERVATIONS, AND MEASUREMENTS

The in-life procedures, observations, and measurements listed below will be performed for all animals.

In-life Assessments

Parameter	Frequency (minimum required)	Comments
Mortality	At least twice daily (morning and afternoon) during the study	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.
Clinical Observations	Once daily on Days 1-6 (on Days 1-3 between 3 and 4 hours after dosing)	Animals will be observed within their cage unless necessary for identification or confirmation of possible findings.
Individual Body Weights	On Days 1 (predose) and 6 (prior to necropsy)	Animals will be individually weighed. Terminal body weights will also be collected from animals if found dead or euthanized moribund after Day 1.
Irritation (ears)	Once daily on Days 1-6 (on Days 1-3 within 1 hour after dosing).	According to the numerical scoring system shown below. Furthermore, a description of all other (local) effects will be recorded.

Erythema and eschar formation:

No erythema 0
Very slight erythema (barely perceptible) 1
Well-defined erythema 2
Moderate to severe erythema 3
Severe erythema (beet redness) to eschar formation preventing grading of erythema 4

11. TERMINAL PROCEDURES

11.1. Unscheduled Euthanasia

Moribund animals will be sacrificed by intra-peritoneal injection with pentobarbital (Euthasol® 20% (0.2 mL/animal)). Animals found dead or sacrificed for humane reasons will be subjected to necropsy for gross macroscopic examination (no necropsy will be conducted on the animals of the pre-screen test).Scheduled Euthanasia

11.2. Scheduled Euthanasia, Tissue Collection and Processing

On day 6 of study, each animal will be injected via the tail vein with 0.25 mL of sterile phosphate buffered saline (PBS) containing 20 µCi of ^{3}H-methyl thymidine. After five hours, the animals will be euthanized by intraperitoneal injection with Euthasol® 20% (0.2 mL/animal) and the draining (auricular) lymph node of each ear will be excised. The relative size of the nodes (as compared to normal) will be estimated by visual examination and abnormalities of the nodes and surrounding area will be recorded. The nodes will be pooled for each animal in approximately 3 mL PBS.

Following excision of the nodes, a single cell suspension of lymph node cells (LNC) will be prepared in PBS by gentle separation through stainless steel gauze (maze size: 200 µm, diameter: ± 1.5 cm). LNC will be washed twice with an excess of PBS by centrifugation and the DNA will be precipitated with 5% trichloroacetic acid (TCA) then stored in the refrigerator until the next day.

12. LABORATORY INVESTIGATIONS

12.1. Radioactivity Measurements

On Day 7, precipitates will be recovered by centrifugation, resuspended in 1 mL TCA and transferred to 10 mL of Ultima Gold cocktail scintillation fluid. All radioactivity measurements will be performed using a Packard scintillation counter. Counting time will be to a statistical precision of ± 0.2% or a maximum of 5 minutes whichever comes first. The scintillation counter will be programmed to automatically subtract background and convert Counts Per Minute (CPM) to Disintegrations Per Minute (DPM).

13. ANALYSIS

DPM values will be presented for each animal and for each dose group. A mean Stimulation Index (SI) will be calculated for each group using the individual SI values. The individual SI is the ratio of the DPM/animal compared to the DPM/vehicle control group mean.

If the results indicate a SI ≥ 3, the test item should be regarded as a skin sensitizer.

In case of borderline results, statistical analysis may be performed to determine the dose response relationship and pair wise comparisons between dose groups versus negative control. The methods used will be specified in the raw data and report.

The EC3 value (the estimated item concentration that will give a SI=3) may be determined if possible, based on the dose response relationship or calculated using linear interpolation (reference 1).

If it is not possible to determine the EC3 value, additional groups of animals may be treated. This will be done in consultation with the Sponsor and will be confirmed by study plan amendment.

The results can be evaluated according to the:

- Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (including all amendments).
- Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of items and mixtures (including all amendments).

Classification of results

	UN-GHS 2017; EC-CLP 2008	EC Hazard statement
SI < 3	No sensitizer	
SI ≥ 3	Cat 1 Skin sensitizer EC3 value ≤ 2%: sub-category 1A EC3 value > 2%: sub-category 1B	H317: May cause an allergic skin reaction

14. COMPUTERIZED SYSTEMS

The following critical computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Critical Computerized Systems

System name	Description of Data Collected and/or Analyzed
Deviation Information Library	Deviations
Share Document Management System	Reporting
Docusign	Collection of 21 CFR Part 11 compliant signature
REES Centron	Temperature and Humidity (Animal and Laboratory facilities) Data Collection
Quantasmart	Radioactivity measurements Data Collection

15. REGULATORY COMPLIANCE

The study will be performed in accordance with the OECD Principles of Good Laboratory Practice as accepted by Regulatory Authorities throughout the European Union, United States of America (FDA and EPA), Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

16. QUALITY ASSURANCE

16.1. Test Facility

The Test Facility Quality Assurance Unit (QAU) will monitor the study to assure the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with Good Laboratory Practice regulations. The QAU will review the Study Plan, conduct inspections at intervals adequate to assure the integrity of the study, and audit the Final Report to assure that it accurately describes the methods and standard operating procedures and that the reported results accurately reflect the raw data of the study.

17. AMENDMENTS AND DEVIATIONS

Changes to the approved Study Plan shall be made in the form of an amendment, which will be signed and dated by the Study Director. Every reasonable effort will be made to discuss any necessary Study Plan changes in advance with the Sponsor. The Study Director will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

18. RETENTION AND DISPOSITION OF RECORDS, SAMPLES, AND SPECIMENS

All applicable study-specific raw data, electronic data, documentation, Study Plan, retained samples and specimens, and Final Reports will be archived by no later than the date of Final Report issue. All materials generated by Charles River from this study will be transferred to a Charles River archive. At least 2 year after issue of the Final Report, the Sponsor will be contacted.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Study Plan, Study Plan amendments, and deviations
- Study schedule
- Study-related correspondence
- Test system receipt, health, and husbandry
- Test item receipt, identification and preparation
- In-life measurements and observations

19. REPORTING

A comprehensive Draft Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the study.

The Sponsor will receive an electronic version of the Draft Report. The Final Report will be provided in Adobe Acrobat PDF format (hyperlinked and searchable). The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation.

Reports should be finalized within 6 months of issue of the Draft Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the report will be finalized by the Test Facility unless other arrangements are made by the Sponsor.

20. JUSTIFICATIONS AND GUIDELINES

20.1. Justification of Vehicle

The vehicle was chosen from the vehicles specified in the test guideline (in order of preference): Acetone/Olive oil (4:1 v/v), N,N-dimethylformamide, methylethylketone,

propylene glycol, dimethylsulfoxide and 1% Pluronic© L92 in Elix water (in case an aqueous vehicle is suitable).

The vehicle was selected on the basis of maximizing the solubility based on trial preparations performed at Charles River Den Bosch and on information provided by the Sponsor. Trial preparations were performed to select the suitable vehicle and to establish a suitable formulation procedure. These trials were not performed as part of this study and these preparations were not used for dosing. Raw Data of these trials will be retained by the Test Facility.

20.2. Justification of Test System and Number of Animals

At this time, studies in laboratory animals provide the best available basis for extrapolation to humans and are required to support regulatory submissions. Acceptable models that do not use live animals currently do not exist.

The CBA/J mouse was chosen as the animal model for this study as recognized by international guidelines as a recommended test system (e.g. OECD, FDA, MHLW). The test method and number of animals are based on the test guidelines.

The results of a reliability test with three concentrations of hexylcinnamaldehyde in acetone/olive oil (4:1 v/v), performed not more than 6 months previously and using the same materials, animal supplier, animal strain and procedures will be summarized in the report. For both scientific and animal welfare reasons, no concurrent positive control group will be included in the study. An extensive data base is available with reliability checks performed each half year during at least the recent 9 years showing reproducible and consistent positive results.

20.3. Justification of Route and Dose Levels

Dose route and dose concentrations are in compliance with the OECD test guidelines for LLNA studies.

20.4. Guidelines for Study

The design of this study was based on the study objective(s), the overall product development strategy for the test item, and the following study design guidelines:

- OECD Guideline 429. *Skin Sensitization: Local Lymph Node Assay,* July 2010
- EC No 640/2012 Part B. *Skin Sensitization: Local Lymph Node Assay,* July 2012
- EPA Health Effects Test Guideline OPPTS 870.2600. *Skin Sensitization,* March 2003

21. ANIMAL WELFARE

This study plan was reviewed and agreed by the Animal Welfare Body of Charles River Laboratories Den Bosch B.V. within the framework of Appendix 1 of project license AVD2360020172866 approved by the Central Authority for Scientific Procedures on Animals (CCD) as required by the Dutch Act on Animal Experimentation (December 2014).

Animals showing pain, distress or discomfort, which is considered not transient in nature or is likely to become more severe, will be sacrificed for humane reasons based on OECD

guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (ENV/JM/MONO/ 2000/7).

By approving this study plan, the Sponsor affirms that this study is required by a relevant government regulatory agency and that it does not unnecessarily duplicate any previous experiments.

In the interest of animal welfare and to minimize any testing likely to produce severe responses in animals, a weight of evidence analysis will be performed prior to the start of this study. All available information will be evaluated (e.g. existing human and animal data, literature, item data supplied by the Sponsor, analysis of structure activity relationships (SAR), physicochemical properties and reactivity (pH, buffering capacity)).

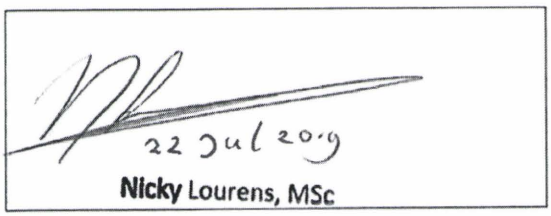
Performance of the in vitro test battery was considered but it was judged that the test battery was not able to fulfil the regulatory requirements. Therefore is was considered that there was need to perform the LLNA.

22. REFERENCES

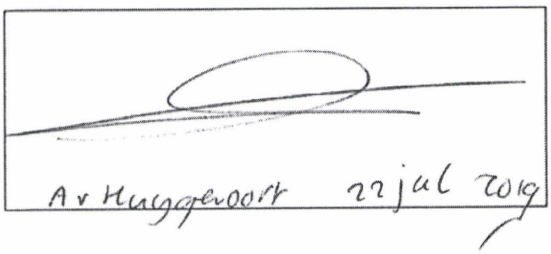
Ref. 1 Basketter, A. et al., A comparison of statistical approaches to the derivation of EC3 values from local lymph node assay dose responses, J. Appl. Toxicol. 19, 261-266 (1999).

TEST FACILITY APPROVAL

The signature below indicates that Test Facility Management approves the Study Director identified in this Study Plan and management's responsibility to the study as defined by the relevant GLP regulations.



The signature below indicates that the Study Director approves the study Study Plan.



SPONSOR APPROVAL

The Study Plan was approved by the Sponsor by e-mail on the date designated below. The correspondence giving approval will be archived, as appropriate with other Sponsor communications.

17 July 2019
Date of Sponsor Approval

ATTACHMENT A

Distribution List

Electronic copies will be supplied unless otherwise specified below.

Version	**Recipient**	
Original	Study Director	
1 Copy	Sponsor Representative / Study Monitor	
1 Copy	QAU / Management	
1 Copy	Section AFC	Heeren, M;
1 Paper copy	Coordinating Biotechnician	Altepost, J;

DEVIATIONS

All deviations that occurred during the study have been authorized/acknowledged by the Study Director, assessed for impact, and documented in the study records. All study plan deviations and those SOP deviations that could have impacted the quality or integrity of the study are listed below. None of the deviations were considered to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

Induction Main Study

- Inadvertently, a 40% test item concentration was used in the main study instead of a 50% test item concentration that was selected based on the results of the pre-screen test. This study plan deviation is considered not to have affected the integrity of the study because this concentration was not relevant for the outcome of the study.